

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of the Claims

Claims 1-22 (Cancelled)

Claim 23 (Currently Amended): A process for the ~~preparation of~~ preparing an agglutination reagent for rapid and early detection of detecting typhoid comprising:

- (a) preparing ~~an a~~ a polyclonal-monospecific antibody specific to *Salmonella* typhi;
- (b) preparing a latex particle suspension; and
- (c) coating ~~of a~~ latex particle with ~~the said~~ a polyclonal-monospecific antibody specific to *Salmonella* typhi;

wherein said polyclonal-monospecific antibody specific to *Salmonella* typhi is prepared according to a method comprising:

- (i) ~~cloning a Flagellin gene sequence specific to Salmonella typhi, expressing said Flagellin gene sequence by recombinant DNA technology thereby expressing a recombinant protein, followed by purifying said recombinant protein by affinity chromatography thereby forming a purified recombinant protein, raising a hyper immune sera against said a purified recombinant protein encoded by a Flagellin gene specific to Salmonella typhi in an animal, and~~
- (ii) ~~separating an said polyclonal-monospecific antibody (immunoglobulin) fraction from said hyper immune sera by precipitating in an ammonium sulphate, suspending in a 50 mM phosphate buffer of pH 7.2, and dialyzing;~~

wherein said latex particle suspension is prepared according to a method comprising:

- (i) mixing 1% carboxylated latex particles of size 0.88 to 0.90 μm and a 40 mM 2-N morpholinoethane sulphonic acid (MES) buffer of pH 5.5 to 6.0 in a ratio of 1:1 ~~on a vortex mixer for about 60 seconds, centrifuging at 10,000 rpm for 10-12 minutes at about 4°C, followed by washing twice with a 20 mM MES buffer of~~

pH 5.5 at 10,000 rpm for 10-12 minutes at about 4°C, sonicating by a tip sonicator at about 5 watts for 60-120 seconds, thereby forming a sonicated-washed latex particle; and

- (ii) ~~adding drop wise a freshly prepared solution of a 0.1 M 1-ethyl-3 (3-dimethyl-amino propyl) carbodiimide hydrochloride (EDC) in a 20 mM MES buffer of pH 5.5 to said sonicated-washed latex particles obtained from step (i) above in a ratio of 1:1 while vortexing slowly thereby forming a suspension, rotating the suspension slowly end-over-end for about 3 hours at a temperature of 20-25°C, washing thrice with a 20 mM MES buffer (pH 5.5); and followed by~~

- (iii) sonicating said suspension by a tip sonicator for 60-120 seconds at about 5 watts; and

wherein said latex particle is coated by according to a method comprising:

- (i) adding-reacting 0.6-1.0 mg per ml of said polyclonal-monospecific antibody (immunoglobulins)-fraction to-with said suspension-washed latex particle thereby forming an antibody-particle mixture, rotating said antibody-particle mixture end-over-end for 18-20 hours at a temperature of about 20-25°C thereby forming a solution comprising an-a polyclonal-monospecific antibody coated latex particle,
- (ii) stopping the reacting step (i) coating-reaction-by adding 1M glycine (pH 11.0) taken in quantity of 0.06 ml per ml of said solution, followed by centrifugation at 10,000 rpm for 10-12 minutes at a temperature of about 4°C, and
- (iii) washing thrice-said antibody coated latex particle polyclonal-monospecific with a washing buffer comprised of 50 mM glycine, pH 8.5; 0.03% surfactant and 0.05% sodium azide, suspending in a storage buffer to a final concentration of 1%, sonicating for about 60 seconds at about 5 watts and storing at 4°C.

Claim 24 (Currently Amended): An agglutination reagent for rapid and early detection of typhoid, comprising of 1% a carboxylated latex particles coated with an antibody specific to *Salmonella* typhi, suspended in storage buffer.

Claim 25 (Previously Presented): The agglutination reagent as claimed in claim 24, wherein the size of the said latex particles is 0.88 to 0.90 μm .

Claim 26 (Previously Presented): The agglutination reagent as claimed in claim 24, wherein the said storage buffer is comprised of 50 mM glycine pH 8.5, 1.0% bovine serum albumin, 0.03% surfactant, 0.1% sodium azide and 0.01% thimerosal.

Claim 27 (Currently Amended): The agglutination reagent for rapid and early detection of typhoid as claimed in claim 24, wherein ~~the said antibody is the~~ an immunoglobulin fraction of the a hyper immune sera raised in said animals against the a recombinant protein encoded expressed by cloning of a Flagellin gene sequence specific to *Salmonella* typhi by recombinant DNA technology, and wherein said storage buffer is a suspended in 50 mM phosphate buffer.

Claim 28 (Withdrawn): A kit for rapid and early detection of typhoid comprising 1% agglutination reagent as claimed in claim 24 suspended in storage buffer, glass slides, droppers, wooden sticks and positive and negative controls.